

The University of Chicago

MICROSPOROGENESIS AND CYTO-
KINESIS IN ASIMINA
TRILOBA

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1934

By
JOHN FLOWERS LOCKE

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE BOTANICAL GAZETTE
Vol. 98, No. 1, 1936

The University of Chicago

MICROSPOROGENESIS AND CYTO-
KINESIS IN ASIMINA
TRILOBA

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY

1934

By
JOHN FLOWERS LOCKE

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE BOTANICAL GAZETTE
Vol. 98, No. 1, 1936



MICROSPOROGENESIS AND CYTOKINESIS IN ASIMINA TRILOBA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 474

JOHN FLOWERS LOCKE

(WITH PLATE I)

Introduction

The North American pawpaw, *Asimina triloba* (L.) Dunal, occurs throughout nearly the whole of the eastern half of the United States, exclusive of the New England states.

Little cytological work has been reported on the two genera of the Anonaceae, *Asimina* and *Anona*, which occur in the United States. VOIGT (8) investigated the structure and development of the peculiarly ruminated endosperm in *Uvaria lowii*, and stated that the other members of the family show no marked differences in either structure or development. NICOLosi-RONCATI (4, 5) reported on the formation of the endosperm and the structure of the ovule in *Anona cherimolia*. HERMS (1) described a few stages in the early development of the megagametophyte in *Asimina triloba*. SAMUELS-SON (7) reported that microspore formation in *Aristolochia clematitis* is by successive constriction, that the same type of constriction occurs in *Asimina*, and discussed the systematic significance of such a type of microspore formation.

Since none of these reports deal with meiosis and the number of chromosomes, a study has been made of nuclear behavior during meiosis in *Asimina triloba*. On examining the material of *Asimina*, a peculiar type of cytokinesis was noticed; and since the heterotypic prophase of the meiotic divisions showed little of significance, this investigation has concerned itself mainly with the type of cytokinesis occurring in the divisions of the pollen mother cells in the formation of microspores.

Materials and methods

Material of *Asimina* was collected in the spring of 1931 near Mississippi State College and in 1933 and 1934 in the region of Smith,

Indiana. Most of the material was killed in the field. The first step in the preparation of the material was to remove the sepals and petals from the floral buds. Good penetration was obtained when the buds were dropped in Carnoy's solution for a few seconds before being placed in the fixing solution. For the earlier stages good results were obtained by the use of a suction pump immediately following immersion of the buds in the fixing solution.

Several fixing agents were used, among them various modifications of Navashin's solution, Flemming's medium and stronger solutions, chromo-acetic-osmic solution, Carnoy's solution, and corrosive sublimate. The modifications of Navashin's solution gave the most satisfactory results.

Sections were cut from 4 to 12 μ , those about 6 μ proving most satisfactory for the study of both nuclear and cytoplasmic division. The preparations used for the study of meiosis were stained in iron-alum haematoxylin, while those used in the study of cytokinesis were counterstained with orange G. Owing to the strong affinity of the mother cell wall for the crystal violet, Flemming's triple stain proved unsatisfactory for preparations designed to show cell division.

Observations

RESTING NUCLEUS TO DIAKINESIS

In the resting condition the pollen mother cells are polyhedral in shape. Usually two pollen mother cells, separated by a single layer of sterile cells, occur across the diameter of the locule. The nuclei now have a diameter of approximately 8 μ . The strands of the reticulum are thin, and where they cross and anastomose, the chromatin is aggregated in thick masses. There is usually one large deeply staining nucleolus, rarely two of unequal size.

As the prophases begin, the nuclei of the mother cells enlarge rapidly, the chromatin network becomes more threadlike, the aggregations spread out along the threads, and a smoother and more uniform appearance is gradually assumed. Soon after these changes occur the threads pair side by side.

The synizetic condition now ensues; but, contrary to the usual condition, in which the synizetic knot lies on the side of the nucleus closest to the mother cell wall, in *Asimina triloba* the knot is on the

side of the nucleus farthest from the wall. The nucleolus is commonly not in contact with the synizetic knot, but lies free within the nuclear cavity. It is always spherical at this time, and may show small peripherally arranged vacuoles. During the changes just described the nuclei have enlarged gradually and continue to do so until at pachytene they have a diameter of approximately $16\ \mu$.

Following synizesis, the chromatin threads, consisting of double strands closely twisted about each other, begin to spread throughout the nuclear cavity. In some cases this association was so close that their double nature could not be detected, but at other places the twisting of the strands was clearly evident.

As the pachytene condition begins, the threadlike mass continues to loosen and spreads throughout the entire nuclear cavity. During early pachytene there is little evidence of the double nature of the strands; but as they pass into diplotene, the paired elements become less closely associated. The threads continue to shorten and assume the typical form and scattered arrangement of bivalent chromosomes which characterize diakinesis. Counts made of bivalent chromosomes at diakinesis indicated a haploid number of nine, although it is not possible to make a definite statement concerning their number. During the anaphases of the second meiotic division nine chromosomes were observed passing to the poles of the two daughter cells, thus tending to confirm this number as being the correct count.

CYTOKINESIS

FIRST DIVISION.—Following diakinesis, the mother cell wall continues to thicken uniformly until at the time the microspores are formed it has a thickness of approximately $6\ \mu$. The spherical pollen mother cells lie free in the anther locule. During diakinesis, the nuclear membrane disappears and the fibers of the diarch spindle, which appears at about this time, become attached to the chromosomes. As the chromosomes pass toward the poles, the mother cell becomes elongated in the direction of the axis of the first division. Occasionally the mother cells begin elongation before the end of the prophase. During interkinesis, the chromatin material of the daughter nuclei does not become reorganized into a definite reticu-

lum, but assumes the form of spherical, irregular, or threadlike masses (figs. 1-5, 7-9). The daughter nuclei are discoid and usually somewhat flattened on the side toward the region of the first cytoplasmic constriction (figs. 3-5, 7-9). After the daughter nuclei have been formed, the spindle spreads out until it extends almost across the mid-plane of the cell (fig. 2). The spindle fibers are thicker and more irregularly arranged than during the anaphases; and as cytokinesis occurs, they gradually become more granular.

The first evidence of cytokinesis is the appearance of a constriction in the equatorial region and in a plane approximately equidistant from the daughter nuclei (fig. 2). The furrow is usually initiated after the daughter nuclei have been formed; in some cells, however, the furrow is initiated during the anaphases of the first meiotic division. When the furrow first begins to form it is narrow and pointed, but soon thickens considerably (fig. 3). The advancing edge of the furrow becomes broad and round, giving the cell a two-lobed appearance, and from the time it is initiated it is completely filled with material in close apposition to the mother cell wall.

In no cell observed was there any indication of a cell plate prior to the initiation of the furrow; but when a cytoplasmic isthmus of approximately one-third the diameter of the mother cell remains between the advancing edges of the furrow, an inconspicuous cell plate may be formed in the mid-region of the spindle (figs. 3-5). This condition occurred in a number of the cells observed in this stage of development; in others there was no indication of such a structure.

The furrow continues to deepen; and when the isthmus remaining is about one-fifth the diameter of the mother cell in the direction of the constriction, the cell plate, if formed, has completely disappeared (figs. 6-8). Thus the cell plate formed here is an evanescent structure and plays no part in the furrowing process. The spindle fibers have now disappeared also, and nothing was observed to indicate that they play a part in the formation of the developing plasma membranes, as suggested by FARR (2).

Within a short time the cytoplasmic isthmus is completely severed by the constriction of the plasma membrane, and the wall of the

mother cell forms a continuous structure between the two daughter cells (fig. 9). The wall which develops between the daughter cells is about three times as thick as the mother cell wall surrounding the two cells and, as shown by microchemical tests, consists of callose.

Since there is no definite correspondence between nuclear phases and stages in the furrowing process, it is not possible to arrange a series of stages in cytokinesis on the basis of nuclear changes. In the majority of cells the first cytoplasmic division is completed before the second nuclear divisions occur, although occasionally the latter may occur during the process of the first constriction.

SECOND DIVISION.—The spindles of the second nuclear division figures now form and are usually parallel with one another and perpendicular to the axis of the spindle of the first division. Figure 10 shows a cell during the anaphases of the second nuclear division with nine chromosomes passing to each pole. Following this the spindles disappear, and no suggestion of a cell plate was observed in any of these division figures.

The furrow of the second cytoplasmic division begins along the inner periphery of the daughter cells adjacent to the wall of the first division (fig. 11). It proceeds to about one-third the diameter of the cells and now begins to appear along the outer peripheral wall (fig. 12). Furrowing then proceeds on all sides at about the same rate, and constriction is completed at a point closer to the outer peripheral wall than to the wall which comes in following the first constriction furrow (fig. 13). In figure 14 a condition is shown in which one of the daughter cells is dividing more rapidly from the outer wall, and constriction of one of the daughter cells will be completed closer to the wall of the first division than to the mother cell wall. Usually the second divisions are completed at approximately the same time, but occasionally one of the daughter cells may divide before the other. One of the daughter cells may undergo the second cytoplasmic division before second nuclear division has been initiated in the sister cell (fig. 15).

The two successive divisions of the pollen mother cell form four microspores which are quadrilaterally arranged (fig. 16). The locules of the anther are of such small diameter that one quartet of

microspores fills the entire space. After the second division is complete, the nuclei lie close to the plasma membrane of the microspores, but not in contact with it. After a time they become centrally placed.

DEVELOPMENT OF POLLEN GRAIN

The young microspores, with dense granular cytoplasm, measure approximately $30\ \mu$ in diameter (fig. 16). They soon begin to enlarge, apparently with little increase, if any, in the amount of cytoplasm at this time. With the increase in size the vacuoles enlarge and the cytoplasm remains for a time distributed throughout the cell. At the time the microspore has reached its full size, the cytoplasm lies mainly at one side, having one or a few large vacuoles in the central portion of the cell (fig. 17). During enlargement of the microspore its wall increases in thickness, developing a pectose intine layer and an exine layer of undetermined composition. After a time the cytoplasm begins to increase in volume; simultaneously the vacuoles become smaller and more numerous, until the cytoplasm of the microspores finally appears densely alveolar (figs. 18-21).

The nucleus of the microspore now divides, thereby forming the generative and the tube nuclei of the young microgametophyte (fig. 21). This division may occur before the cytoplasm has become densely alveolar (fig. 20). The generative nucleus is smaller than the tube nucleus, and it, with a small amount of cytoplasm, is set off from the tube cell by a membrane. When the binucleate condition of the microgametophyte occurs the tapetal tissue has disappeared and the anthers have opened for the shedding of pollen.

Discussion

FARR (2) gives an extensive review of the literature dealing with cytokinesis in pollen mother cells. His results tend to indicate that the divisions of the pollen mother cells, in most angiosperms, are brought about by furrowing rather than by cell plates. In general, bipartition is common to the monocotyledons and quadripartition to the dicotyledons, but intermediate types of microspore formation have been reported for a few scattered forms. Some of those which are similar to *Asimina* will be discussed briefly.

ROSANOFF, in his work on *Acacia paradoxa*, gives two figures (6, figs. 24, 25) showing bipartition of the pollen mother cells by furrowing. After completion of the first division, the furrowing appears along the periphery of the daughter cells and later along the wall of the first division furrow. Cell plates are not shown, and he does not discuss the process of cytokinesis. From these two figures it appears that the division in *A. paradoxa* is similar to that which occurs in *Asimina triloba*. It differs, however, in that in the former the furrow of the first division appears first along the outer periphery of the daughter cells, while in *Asimina* it appears first along the inner periphery of the daughter cells adjacent to the wall of the first division furrow.

In his study of *Magnolia*, FARR (3) reported that the first cytoplasmic division of the pollen mother cells begins during interkinesis and continues until the isthmus of cytoplasm which remains is about one-half the diameter of the mother cell. Division is now arrested, the fibers disappear, and the second nuclear divisions occur. During interkinesis, the furrow of the first division may almost disappear. The furrow of the second division appears at the periphery of the cell and at right angles to the axis of the spindle of the first division. The spindle of the first nuclear division has now reappeared. The furrow of the second division deepens more rapidly than the furrow of the first division so that both constrictions are completed at approximately the same time. There was no indication of a true cell plate after the initiation of the constriction. FARR suggested that furrowing might have been brought about by attraction between the nuclear and cytoplasmic membranes.

FARR (2) observed that the spindle fibers come in contact with the plasma membrane at the advancing edge of the furrow, and suggested the possibility of their being used in the formation of the developing plasma membranes. The spindle fibers in *Asimina* were not observed to come in contact with the plasma membrane as the furrow advanced.

SAMUELSSON (7) reported a type of cytokinesis in *Anona cherimolia* that is similar to that in *Asimina triloba*. Division is by successive constriction, and the mother cell is divided into two daughter cells. The furrow begins during the telophases of the first nuclear

division and is completed after the second nuclear divisions have occurred. During the metaphases of the second nuclear division, the cells are connected by a narrow bridge of cytoplasm. The second division takes place in the same manner as the first. *Asimina* differs from *Anona* in that there is not so close a correlation between nuclear phases and stages in the furrowing process in the former as occurs in the latter. The second division in *Asimina* differs from the second division in *Anona* in that (as already shown) it does not agree completely with the first division as SAMUELSSON described for *Anona*. SAMUELSSON thought that the type of division that occurred in *Anona* was more closely related to the monocotyledonous than to the dicotyledonous type.

SAMUELSSON reported a similar type of division in *Aristolochia clematitis*. Immediately after the first cytoplasmic division the intervening wall thickens greatly. This was observed to be true in *Asimina*. In *Aristolochia* the spindles of the second division may not always be parallel to one another and perpendicular to the axis of the spindle of the first division. The four microspores may be arranged in a straight line as a result of the spindles of the second division being in the same plane as the spindle of the first division. In some cells he reported one of the spindles of the second division to be parallel with the spindle of the first division and the other perpendicular to it, the two spindles of the second division being in the same plane.

HERMS observed the pollen mother cells in *Asimina triloba* to be differentiated in early October, and stated that they remain in this condition until the middle of January. He reported that on March 10 the pollen mother cells begin to enlarge, and by April 14 a bridge of tissue is formed between the microsporocytes. Spore tetrads are formed by April 21. By May 5 pollen is ready to be shed. Similar definite dates were given for the stages in development of the endosperm and megagametophyte. My own observations indicate that it is not possible to associate stages of development with precise dates because of the great variation in the different buds used for study. Floral buds were collected in late March in which pollen mother cells had not become differentiated. Also, in some collected as late as May 1, microspores had not been formed.

Summary

1. During meiosis the chromatin material of the pollen mother cells undergoes parasynaptic association.
2. At synizesis the chromatic knot lies on the side of the nucleus farthest from the mother cell wall.
3. The haploid number of chromosomes is apparently nine.
4. Cytokinesis of the pollen mother cells is by successive constriction. Evanescent cell plates may occur in the first division.
5. In general the first cytoplasmic division is completed before the second nuclear divisions occur.
6. In the second cytoplasmic division the furrow appears first on the side of the daughter cells adjacent to the wall of the first division, and proceeds outward for a time before it appears at the outer periphery of the cell.
7. The young microspores are relatively small and have a quadrilateral arrangement as a result of the spindles of the second division being perpendicular to the axis of the first division and in the same plane with it.
8. Before being shed, the microspores develop into relatively large binucleate microgametophytes which are completely filled with dense, alveola-like cytoplasm.

The writer expresses appreciation to Dr. J. M. BEAL for suggestions and constructive criticism throughout the course of this investigation.

DEPARTMENT OF BOTANY
MISSISSIPPI STATE COLLEGE
STATE COLLEGE, MISSISSIPPI

LITERATURE CITED

1. HERMS, W. B., Contribution to the life history of *Asimina triloba*. Ohio Nat. 8:211-217. 1907.
2. FARR, C. H., Cytokinesis of the pollen-mother-cells of certain dicotyledons. Mem. New York Bot. Gard. 6:253-317. 1915.
3. ———, Cell division by furrowing in *Magnolia*. Amer. Jour. Bot. 5:379-396. 1918.
4. NICOLSI-RONCATI, F., La formazione dell'endosperma nell' *Anona Chermolia* L. Bull. Soc. Bot. Ital. no. 4. 115-117. 1903.

5. NICOLOSI-RONCATI, F., Sviluppo dell' ovulo e del seme nella *Anona Cherimolia* Mill. Atti Accad. Gioenia Sci. Nat., Catania 18: Mem. II. 1-26. 1905.
6. ROSANOFF, S., Zur Kenntniss des Baues und der Entwicklungsgeschichte des Pollens der Mimoseae. Jahrb. Wiss. Bot. 4:441-450. 1865.
7. SAMUELSSON, G., Über die Pollenentwicklung von *Anona* und *Aristolochia* und ihre systematische Bedeutung. Svensk Bot. Tidskr. 8:181-189. 1914.
8. VOIGT, A., Untersuchungen über Bau und Entwicklung von Samen mit rudimentierten Endosperm aus den Familien der Palmen, Myristicaceen, und Anonaceen. Ann. Jard. Bot. Buitenzorg 7:151-190. 1888.

EXPLANATION OF PLATE I

FIG. 1.—Pollen mother cells showing first nuclear division.

FIGS. 2-9.—Pollen mother cells showing successive stages in first cytoplasmic division.

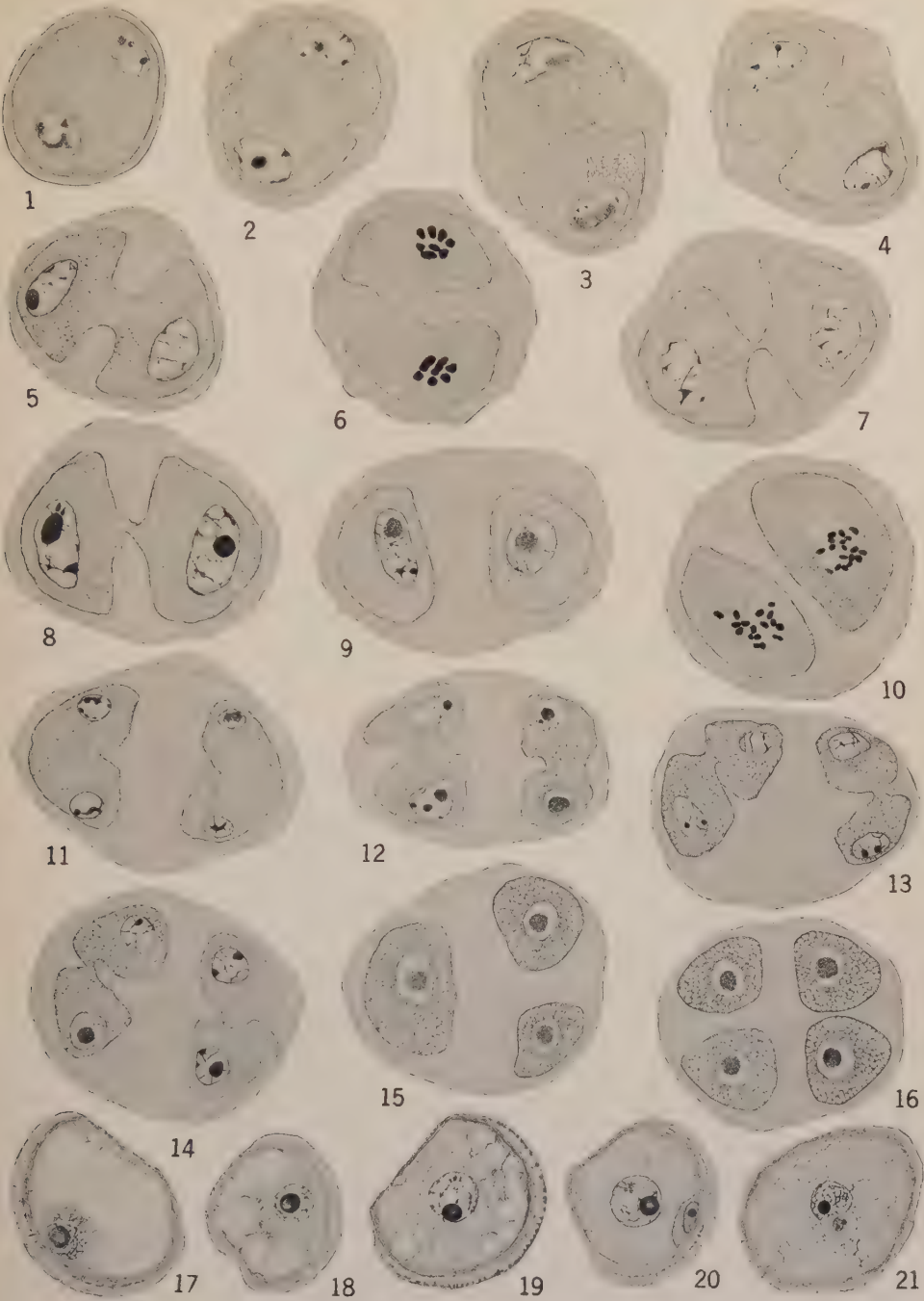
FIG. 10.—Daughter cells in early anaphases of second nuclear divisions with nine chromosomes passing to each pole.

FIGS. 11-14.—Daughter cells showing successive stages in second cytoplasmic division. Fig. 14, unusual condition in one daughter cell in which the furrow has proceeded more rapidly from outer periphery toward wall of first division.

FIG. 15.—One daughter cell which has undergone second cytoplasmic division before the second nuclear division of sister cell has been initiated.

FIG. 16.—Quartet of microspores quadrilaterally arranged.

FIGS. 17-21.—Successive stages in development of pollen grain from mature microspore.



LOCKE on ASIMINA

